REMARKS

The Present Invention

The present invention is directed to an amplification based method for producing a mammalian promoter containing siRNA expression cassette. The mammalian promoter is capable of transcribing an siRNA molecule in mammalian cells. The method comprises three steps. In a first step, one strand of a double-stranded mammalian promoter sequence in an amplification reaction mixture is treated with a first oligonucleotide primer. This first primer is complementary to the 5' end of the mammalian promoter sequence. In a second step, the other strand of the double-stranded mammalian promoter sequence in the amplification reaction mixture is treated with a second oligonucleotide primer. This second primer is complementary to the 3' end of the mammalian promoter sequence and further comprises a sequence which is complementary to a sequence encoding either the sense or antisense strand of a siRNA molecule and a terminator sequence. As shown in Figure 1, this further sequence is located on the 5' end of the second primer. In a third step, amplification is performed and repeated a sufficient number of times to amplify the mammalian promoter sequence. It is clear from the claim language that the primers used in this method are complementary to the promoter sequence and amplify the promoter sequence. It is equally clear from the claim language that the primers do not contain the complete promoter sequence. In addition, it is clear that the amplified product will contain a promoter sequence on only one end of the double-stranded amplified product. These aspects of the invention are not taught or suggested in the prior art.

Rejection Under 35 U.S.C. § 103(a)

The Examiner has maintained her rejection of claims 1-9, 17, and 19-23 under 35 U.S.C. 103(a) as being unpatentable over Engelke et al. (US 2003/0148519) in view of Livache et al. (US 5,795,715). The Examiner relies on Engelke et al. for teaching 1) a U6 promoter-containing siRNA expression cassette further comprising a transcription termination sequence, constructed by an expression vector construct and a method of transfecting the cassette into mammalian cells

and 2) PCR as a method for amplifying a segment of a genetic sequence without cloning or purification. The Examiner relies on Livache et al. for disclosure of the actual utility of PCR for generating expression cassettes. According to the Examiner, Livache et al. teach a method of producing a double-stranded RNA expression cassette containing a promoter via a PCR-based method by integrating oligonucleotide primers that are complementary sequences that encompass the sequence of a promoter and the target sequence, wherein the duplex RNA has a defined length. The Examiner notes Livache et al. teach that such method is rapid and inexpensive.

Thus, the Examiner concludes that it would have been obvious to one of ordinary skill in the art at the time the invention was made to use the PCR-amplification method of Livache et al. to make the U6 promoter-containing siRNA expression cassette of Engelke et al. Moreover, according to the Examiner, one of ordinary skill in the art would have been motivated by increased efficiency and reduced costs to replace the molecular cloning method of constructing a U6 promoter-containing siRNA expression cassette of Engelke et al. with the PCR-based amplification method of Livache et al.

While addressing Applicants' previous response, the Examiner concedes that Livache et al. do not teach primers that comprise a two-part sequence comprising a promoter sequence and a sense/antisense siRNA strand sequence. In maintaining the rejection, however, the Examiner relies on Livache et al. for teaching that the "PCR-based amplification method is useful in producing a double-stranded RNA expression cassette structure comprising using a two part sequence primer comprising a promoter sequence and a target sequence." The Examiner also relies on Engelke et al. for teaching that the "PCR-based amplification method can produce a desired, double-stranded nucleotide product without extraneous steps of cloning or purification." Thus, the Examiner concludes that "it would have been obvious to one of ordinary skill in the art to make and use a two-part primer sequence that can simultaneously amplify both the promoter sequence and the following target sequence, which can be either the siRNA sense strand sequence or the siRNA antisense strand sequence."

Applicants respectfully disagree and submit that the combined disclosures of Engelke et al. and Livache et al. do not teach PCR amplification of a promoter sequence in which one

primer is complementary to the 5' end of the mammalian promoter sequence, and another primer is complementary to the 3' end of the mammalian promoter sequence and comprises a sequence which is complementary to a sequence encoding either a sense or antisense strand of a siRNA molecule.

First, Livache et al. do not disclose the amplification of a promoter sequence using primers complementary to the promoter sequence. Instead, Livache et al. disclose amplification of a DNA template sequence. The amplification is carried out by using two primers. The first primer is complementary to the 5' end of one of the strands of the DNA template. The second primer is complementary to the 3' end of the other strand of the DNA template. Each primer contains at its 5' end, a promoter sequence of an RNA promoter. See, for example, Col. 3, lines 30-31 and 56-59. Thus, both of Livache et al.'s primers comprise the full promoter sequence at the 5' end of the primers. These primers are not complementary to the 5' or 3' end of a promoter sequence, as recited in the claims. In addition, the primers of Livache et al. are used to amplify the DNA template sequence and not to amplify a promoter sequence. See, for example, Col. 6, lines 12-15. Applicants submit that it is clear from the teachings of Livache et al. that the primers disclosed therein **contain** a full promoter sequence at the 5' end of both primers and that the primers are complementary to the template sequence which is the target sequence for amplification. The amplified nucleic acid in Livache et al. is a double-stranded nucleic acid that contains a promoter sequence at both ends of the double-stranded amplified product and not a single end as in the amplified products of the present invention. Since Livache et al. teaches amplification of a target sequence using primers complementary to the target sequence and designed to include a promoter sequence on the 5' end of the complementary target sequence, Applicants submit that Livache et al. teaches away from using primers that are complementary to a promoter sequence in which one of the primers includes a sequence encoding a sense or antisense strand of a siRNA molecule. Furthermore, Livache et al. does not teach or suggest all of the elements of the claimed subject matter because it does not teach or suggest the primers set forth in the claimed subject matter.

Engelke et al. (in the passages supported in the provisional application) only discuss PCR in the context of the amplification of a target sequence. Engelke et al. do not describe or suggest the amplification of a promoter using the primers set forth in the claimed subject matter. That is, Engelke et al. also does not disclose a first primer complementary to the 5' end of the mammalian promoter sequence and a second primer complementary to the 3' end of the mammalian promoter sequence, in which the second primer includes a sequence encoding a sense or antisense strand of a siRNA molecule. Thus, Applicants submit that Engelke et al. also does not disclose or suggest all of the elements of the claimed subject matter.

Second, although Livache et al. disclose primers that are complementary to the 3' end of target sequences, these 3' end primers do not comprise the sense or antisense strand of a siRNA molecule, as recited in the claims. Thus, Applicants submit that this element is lacking in the teachings of Livache et al. Neither Livache et al. nor Engelke et al. disclose a primer that is complementary to a promoter sequence and *comprises* a sequence which is complementary to a sequence encoding either a sense or antisense strand of a siRNA molecule. Livache et al. teach the use of two primers to prepare a double-stranded DNA of a target nucleic acid. Livache et al. only disclose primers that flank (or are complementary to) the target sequence at the 3' end of each of the stands to be amplified. See, for example, Col. 5, lines 16-22. Livache et al. does not disclose a primer that *comprises* the sense or antisense strand of a siRNA molecule. Even if one were to assume that the target sequence disclosed in Livache et al. could be either the siRNA sense or antisense strand sequence, as the Examiner suggests, the primers merely flank the target sequence and are complementary only to the 3' end of the target sequence, i.e., the primers would amplify the siRNA target sequence. The primers of would not fully comprise the sense or antisense strand of a siRNA molecule, because it is the target sequence, i.e., the Examiner's proposed siRNA target sequence that is amplified. Thus, the combined disclosures of Engelke et al. and Livache et al. do not teach PCR amplification of a promoter sequence in which the first primer is complementary to the 5' end of the mammalian promoter sequence and the second primer is complementary to the 3' end of the mammalian promoter sequence and comprises a sequence which is complementary to a sequence encoding either a sense or antisense strand of a

siRNA molecule. Thus, Engelke et al. and Livache et al. do not teach or suggest all of the elements of the claimed subject matter.

Third, Engelke et al.'s teaching that naked siRNA molecules are expensive to synthesize and have a short life span in cells would not provide motivation to one skilled in the art to replace the molecular cloning method of constructing a U6 promoter-containing siRNA expression cassette of Engelke et al. with the PCR-based amplification method of Livache et al. as asserted by the Examiner. Engelke et al. disclose problems associated with naked siRNA molecules and teaches an alternative – a U6 promoter-containing siRNA expression cassette. The problems associated with naked siRNA which was solved by Engelke et al. would not have motivated one of ordinary skill in the art to modify the method of Engelke et al. because the problems had already been solved. Thus, Applicants submit that there is no motivation to combine the teachings of Engelke et al. and Livache et al. Furthermore, as described above, even if Engelke et al. would have been modified by Livache et al., such modification would not have produce all of the elements of the claimed subject matter. Thus, Applicants submit that the claimed subject matter is not obvious from the teachings of Engelke et al. and Livache et al.

In view of these remarks, Applicants submit that the combined teachings of Engelke et al. and Livache et al. do not render the claimed subject matter obvious. Withdrawal of this rejection is requested.

Rejection Under 35 U.S.C. § 103(a)

The Examiner has rejected claims 30-32 under 35 U.S.C. 103(a) as being unpatentable over Engelke et al. in view of Livache et al., Noonberg et al. (US 5,624,803) and Dietz (US 5,814,500). The Examiner relies on Engelke et al. and Livache et al. for the same disclosures as in the previous obviousness rejection and relies on the same motivation to combine these references. The Examiner further relies on Noonberg et al. for disclosure of "an RNA polymerase III promoter-containing vector for expressing and generating short oligonucleotides such as antisense oligonucleotides and ribozymes, wherein the RNA polymerase III promoter is a human U6 promoter or a human H1 promoter." (cl. 2, 7-12). The Examiner also relies on Dietz

for disclosure of "an RNA polymerase II promoter-containing vector for expressing an antisense oligonucleotide or a ribozyme, wherein the RNA polymerase II is a mammalian U1 snRNA promoter." (claims 1-2, 7-8). Furthermore, the Examiner states that "since both mammalian H1 promoter and U1 snRNA promoter were known to be useful for expression short-length oligonucleotide molecules as taught by Noonberg et al. and Dietz, one of ordinary skill in the art would have been motivated to substitute the mammalian U6 promoter of Engelke et al. for the functionally equivalent mammalian H1 promoter of Noonberg et al. or the mammalian U1 snRNA promoter of Dietz with reasonable expectation of success." Thus, the Examiner concludes that claims 30-32 would have been *prima facie* obvious at the time of filing.

Applicants submit that neither Noonberg et al. nor Dietz cures the deficiencies of the combined teachings of Engelke et al. and Livache et al. Neither of these references disclose PCR amplification of a promoter sequence in which a first primer is complementary to the 5' end of the mammalian promoter sequence and a second primer is complementary to the 3' end of the mammalian promoter sequence and comprises a sequence which is complementary to a sequence encoding either a sense or antisense strand of a siRNA molecule. Thus, Engelke et al., Livache et al., Noonberg et al. and Dietz do not teach or suggest all of the elements of the claimed subject matter. Also, neither of these references provides a proper motivation to combine the teachings of the prior art. Thus, Applicants submit that this rejection fails for the same reasons discussed above.

In view of the above remarks, Applicants submit that the combined teachings of Engelke et al., Livache et al., Noonberg et al. and Dietz do not render the claimed subject matter obvious. Withdrawal of this rejection is requested.

Conclusion

In view of the above remarks, Applicants believe that the present claims satisfy the provisions of the patent statutes and are patentable over the cited prior art. Reconsideration of

the application and notice of allowance are requested. The Examiner is invited to telephone the undersigned if it is deemed to expedite allowance of the application.

Respectfully submitted,

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